

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

Exhibit C

Biocompatibility of lipid-protein-sugar particles containing bupivacaine in the perineurium

Daniel S. Kohane ^{1, 2}

Michael Lipp ²

Ramsey C. Kinney ²

Douglas C. Anthony ³

Noah Lotan ⁴

Robert Langer ²

¹ Dept. of Pediatrics, Massachusetts General Hospital and Harvard Medical School, Boston, MA

² Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

³ Dept. of Pathology, Children's Hospital and Harvard Medical School, Boston, MA

⁴ Dept. of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa, Israel

Abstract

Purpose. The assessment of the perineural biocompatibility of lipid-protein-sugar particles (LPSP). These particles have the potential for improved biocompatibility over some polymeric vehicles, in that LPSP are composed of excipients that occur naturally in the body. We compare the LPSP to poly(lactic-co-glycolic) acid (PLGA) microspheres.

Methods. Male Sprague-Dawley rats were injected with LPSP or PLGA microspheres at the sciatic nerve. At predetermined intervals, tissue reaction was assessed by presence of polymer, difficulty of dissection, and histological description of perineural inflammation. Animals were examined for behaviors believed to be associated with potential nerve injury: touch-evoked agitation and autotomy.

Results. Four days after injection, both types of particle caused inflammatory reactions that were confined to the loose connective tissue. Two weeks after injection, LPSP-injected rats had significantly less polymer residue, a smaller area of inflammation, and less cellularity than the PLGA-injected group. PLGA-injected rats showed foreign body reactions at 8 weeks, and as far out as 7 months after injection. Some animals in the PLGA group showed large particle residues in subcutaneous tissue or tracking down to the knee. Intraneural injection of PLGA microspheres occurred in one case. No animal displayed touch-evoked agitation nor autotomy.

Conclusions. The tissue reaction to LPSP was milder and of shorter duration than that to PLGA microspheres.

Abbreviations

LPSP Lipid-protein-sugar particle

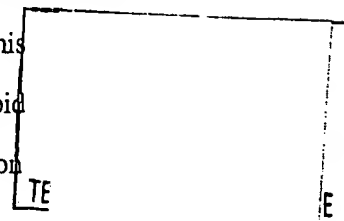
PLGA poly (lactic-co-glycolic) acid

Introduction

Although there have been numerous reports of controlled release technology being used to provide prolonged duration anesthesia, there are few reports on the long-term inflammatory and tissue compatibility sequelae of these methods. This is of special importance when a sustained release vehicle is used for local delivery to sensitive or vital structures (e.g. nerves), particularly if the dwell time of the polymeric device in the target tissue is much longer than the clinical efficacy of the delivered drug. The problem is further exacerbated by the large loads of drug and polymer that typically must be delivered in order to achieve effective and prolonged nerve blockade given the relatively low potency of most conventional local anesthetics (3, 4, 6).

Lipid-protein-sugar particles (LPSP) made of dipalmitoylphosphatidylcholine (DPPC), albumin, and lactose can be loaded with conventional local anesthetics to provide prolonged duration local anesthesia (8). LPSP have two potential advantages over some other types of particles. First, since they are constituted from compounds that are naturally occurring in the body, they might have improved biocompatibility compared to existing polymeric vehicles. Second, LPSP would be expected to degrade more rapidly than some polymeric delivery systems and so their residue would not be expected to be as long lasting.

In this report, we assess the potential for a number of possible adverse reactions to the injected particles. The primary focus of the study was an examination of the biocompatibility of particles in terms of inflammatory response and gross neural injury as assessed by standard histological techniques. We also examine the incidence of "touch-evoked agitation", a problem that has been described with epidural application of particles containing phospholipids. This phenomenon was described in animals that received intrathecal injections of liposomes and phospholipid emulsions containing local anesthetics (18): they appeared in distress when the injected area was palpated. Although this phenomenon is not well understood, it is may be due to a product of phospholipid hydrolysis and to be particularly prominent in phospholipids whose gel-transition



temperatures are close to body temperature (18). Because of the unknown potential for nerve injury from these particles, we also tested the rats for the development of self-mutilation ("autotomy") in the blocked leg (16), a behavior that results from nerve injury and is believed to be pain-related (although this is controversial). PLGA microspheres *per se* do not produce autotomy (17) when injected at the perineurium.

Evaluation of the relative biocompatibility of different controlled release preparations described in the literature is impeded by the marked heterogeneity of experimental designs. Therefore, we compare LPSP to another delivery vehicle that is commonly employed to deliver local anesthetics, microspheres made from high molecular weight poly(lactic-co-glycolic) acid (PLGA) (3, 4, 6, 7, 9, 10, 14, 15), in a blinded study. We have kept as many parameters as possible constant between groups (means and site of drug administration, weight of particle delivered per rat, behavioral observer, dissection and histological techniques and time intervals), and observations were made in a blinded manner. The inflammatory potential of α -hydroxy acids such as PLGA (1, 2, 5, 11, 13) when applied perineurally (6) is well described.

Materials and Methods

Preparation of spray-dried lipid-protein particles (LPSP)

LPSP containing 10% and 0% (w/w) bupivacaine, and PLGA microspheres containing 50% and 0% (w/w) bupivacaine were prepared and characterized as described (8).

Animal Care

Young adult male Sprague-Dawley rats weighing 310 - 420 g each were obtained from Taconic Farms (Germantown, NY), and housed in groups in a 6 AM - 6 PM light-dark cycle. Animals were cared for in compliance with protocols approved by the Animal Care and Use Committee at the Massachusetts Institute of Technology. Rats were only injected once.

Perineural Injection Technique

Nerve block injections were performed via a 20 gauge needle under halothane-oxygen anesthesia as described (8). In brief, each rat was injected with 75 mg of LPSP or microspheres suspended in 0.6 ml of 1% sodium carboxymethyl cellulose, 0.1% Tween 80 (3, 4, 6) with gentle agitation (< 5 sec) in preparation for injection. The presence of nerve block (i.e. proper location of injection) was confirmed (8) in all animals except those injected with blank (no bupivacaine) particles.

Autotomy Scoring

At fixed time intervals, each rat was scored as follows (16). One point was given for mutilation of one or more nails on the hindpaw. An additional point was assigned for each distal (5) and proximal (5) half-digit attacked by the rat. Thus the score could range from 0 to 11. Rats who achieved a score of 11 were sacrificed. Xx? Where is touch-evoked method?

Rat Sciatic Nerve Dissection

Under deep halothane/O₂ general anesthesia, the sciatic nerve was exposed by a longitudinal incision on the lateral thigh, followed by careful separation of the muscles of the anterior and posterior thigh. The wound was extended proximally until the entire area of injection (the area posteromedial to the greater trochanter) was fully revealed. The nerve was then cut above the greater trochanter and at the trifurcation and placed into 4% formaldehyde at 4°C. Intraperitoneal pentobarbital (100 mg/kg) was administered following removal of the nerve.

The dissector was blinded as to which type of particle each rat had been injected with. At the time of dissection, the degree to which the tissues surrounding the nerve were matted together was scored as follows: "0": tissue planes obvious and easily separated, "1": tissue planes obvious but separated with some difficulty, "2": tissue planes effaced and separated with some difficulty, "3": tissue planes completely obliterated, could not separate surrounding tissues from nerve without cutting through them.

Histological Preparations

Embedding, sectioning and staining with hematoxylin/eosin were performed using standard techniques. The amount of inflammation was estimated by cell counting in the most inflamed areas of the sections. The observer was blinded with respect to type of particle. Cell counts were performed on digitized images obtained using light microscopy at 400X. Image size was calculated using a calibration micrometer. The area of inflammation in each dissected nerve was estimated by measuring the long and short axes of the mass and assuming a generally rectangular shape.

Statistical Analysis

Neurobehavioral data are reported as means with standard deviations. Comparisons between groups of such data were made using Student's t-test. Non-parametric data (dissection scores, presence vs. absence of polymer residue) and data that were not normally distributed (area of inflammation) were compared using the Mann-Whitney U-test.

Results

The sciatic nerves of rats that had been injected with LPSP or PLGA microspheres, were removed 4 days ($n = 4$), 2 weeks ($n = 6$), or 7 months ($n = 4$, LPSP; $n = 5$, PLGA) after injection and processed for histology, together with adherent tissues. Fig. 1 shows the histological appearance of a control nerve sciatic nerve. Fig. 2 shows the typical distribution of particles (in this case PLGA microspheres) when injected near the sciatic nerve. In general, neither type of LPSPs nor PLGA microspheres were found within the perineurium. There was no clinical or histological evidence of infection in any of the animals at any timepoint.

Tissue reaction

At four days, all rats injected with PLGA microspheres had large firm, slightly gritty deposits of particle in discrete globules around the nerve. Three out of four rats that received LPSP had visible deposits of particle, which were much smaller than the PLGA deposits and were soft to the touch. There was no statistically significant difference between the PLGA and LPSP groups in any of the parameters shown in Fig. 3. Although both groups had large areas of inflammation and high cell densities on light microscopy, there were marked histological differences (Fig. 4). The PLGA group showed a foreign-body-type granulomatous reaction with multinucleated giant cells surrounding the microspheres (which appear as empty circles 50 to 90 μm across, some with traces of polymer remaining). The LPSP group showed mostly acute inflammation with lymphocytes and macrophages and occasional neutrophils and foamy macrophages. LPSP were not discernible as discrete particles, but as an area of diffuse pink staining. In general (and at all time points examined), the inflammatory response to PLGA microspheres was confined to clearly demarcated pockets. Inflammation from LPSP was found more diffusely throughout the tissues. In some animals in both groups, inflammation also involved adjacent muscle and perineural adipose tissue, with necrotic muscle fibers,

REC

RECEIVED OFFICE

myophagocytosis, and myocyte regeneration – all signs of acute muscle injury. Necrosis was associated with acute inflammation, with neutrophils forming small sterile microabscesses.

Deposits of microspheres were found in all rats injected with PLGA particles two weeks after injection; these were often several millimeters across. A very small amount of residue (approximately 0.5 mm by 3 mm) was found in only one of the rats injected with LPSP. This difference in incidence was statistically significant ($p = 0.005$). PLGA particles were surrounded with a large dense granulomatous foreign body reaction (Fig. 5a) with lymphocytes and macrophages, while LPSPs had a small loose, predominantly lymphocytic infiltrate (Fig. 5b). Microscopic examination of histological preparations from rats injected with PLGA microspheres revealed 4787 ± 1925 cells per mm^2 , which was 3.4 times more ($p = 0.006$) than the 1393 ± 622 cells per mm^2 seen in those injected with LPSP (Fig. 3). The area of inflammation was also much larger in the PLGA group (Fig. 3, $p = 0.01$). One PLGA-treated nerve showed neutrophils and active myositis. In another PLGA-treated rat, a massive cavitory mass was found at the injection site, with a pronounced granulomatous reaction and a large degree of axonal degeneration, on the side of the nerve facing the granuloma.

A third group of animals was sacrificed seven months (210 days) after injection and their sciatic nerves were harvested for histology. All of the dissections of LPSP-injected rats ($n = 4$) were scored as "0". In the PLGA group ($n = 5$) 2 had a score of "1", and one had a score of "2" ($p = 0.08$). No pockets of polymer residue were visible at dissection in either group. On microscopic examination, most samples at this time point were felt to be either entirely normal, or to have slightly increased cellularity with a loose architecture suggesting the possibility of edema, with several lymphocytes. One sample from the PLGA groups showed a small foreign body reaction around a piece of extraneous material. There was no statistically significant difference in cell density between groups, nor did either

represent a significant increase over the cellularity of control nerves (370 ± 38 cells/mm², $p = \text{n.s.}$ vs. both particle groups).

An additional group who received PLGA microspheres was harvested eight weeks after injection ($n = 5$). All five rats showed large amounts of polymer residue. All rats had a dissection score of 2. The histological appearance was comparable to that seen at 2 weeks, with a cell density of 3530 ± 1817 cells/mm².

In order to verify whether the inflammatory reaction to the PLGA-microspheres was due to the polymer or the delivered drug four rats were injected with blank (no bupivacaine) microspheres. Dissection two weeks later revealed polymer residue in 4 of 4 rats, with a median dissection score of 2. The inflammatory response was qualitatively similar to that described with bupivacaine loaded microspheres at the same time point. Four rats that had been injected with blank LPSP were dissected at the same time post-injection. None had any polymer residue ($p = 0.008$ compared to the PLGA group). The dissection score was 0 in all four rats ($p = 0.013$ compared to PLGA group).

Other findings on dissection

In some animals, particles were found at locations where they were not intentionally placed. In the group dissected four days after injection, one of the animals injected with PLGA microspheres had a continuous cord of particles that extended almost to the knee from the site of injection, and two others had visible particle (PLGA) residue tracking up into the subcutaneous tissue. Similar findings occurred in the groups dissected two weeks after injection. In one rat injected with PLGA microspheres, microspheres were found within the nerve, beneath the perineural sheath (Fig. 6 a). In another rat injected with PLGA microspheres a mass of microspheres surrounded by inflammation, measuring 8 mm x 0.4 mm x 2 mm was found 2.5 cm distal to the site of injection along the course of the sciatic nerve, near the knee (Fig. 6b). There were no similar findings in rats injected with LPSP.

Touch-evoked agitation, distress, and autotomy scoring

All rats (PLGA and LPSP groups) were palpated at the site of injection 30 minutes after injection, then 1 hour after block wore off. For the next three days they were palpated twice per day. None of the rats in any of the groups appeared distressed by this maneuver. Furthermore, none had an autotomy score above zero. All the rats appeared well groomed, and continued to gain weight throughout the duration of the experiment.

All of the rats in this study had full recovery of sensory and motor function in the injected extremity when the local anesthetic effect of the microparticles resolved (data not shown), and none had any detectable signs of long-term functional deficits or pain-related behavior.

Discussion

There were striking differences in the long-term inflammatory responses to the LPSP and PLGA particles at two weeks after injection. At two weeks post-injection there was only a mild lymphocytic infiltrate around nerves where LPSP had been injected, and the histology was normal at 7 months. The PLGA microspheres produced an inflammatory response that was consistent with the observations of other investigators (6, 11, 13), with pronounced inflammation at 8 weeks ($n = 5$, data not shown). Traces of the inflammatory reaction to PLGA were seen as far out as 7 months after injection. In general the inflammatory changes seen with both types of particles were contained within discrete pockets within the loose connective tissue, that did not affect the sciatic nerve itself.

Lipid-protein particles were mostly gone from the site of injection by 2 weeks after injection, while PLGA microspheres were present in large quantity. Polymer microspheres were still present in large amounts at 8 weeks ($n = 5$, data not shown). Thus the ratio of duration of therapeutic effect to duration of polymer residue is better for the LPSP. Furthermore, since the LPSP do not entail as protracted a presence of a foreign body, they would be more suitable for repeated injections, particularly at the same site. Although the long-term biocompatibility of the LPSP was superior to that of the PLGA microspheres, it is unlikely that this was due to the fact that the excipients were naturally occurring in the human body. This is shown by the fact that there was marked inflammation at 4 days post-injection. Instead, the improved long-term biocompatibility is likely to be due the much shorter dwell time of the particle in the tissue.

Any material that is injected blindly (i.e. not under direct visualization) into the body has the potential for being injected at a site other than the intended target, or of being injected at the correct location but being pushed away by the force of injection, or of migrating even if deposited at the correct location. These possibilities were demonstrated in the rat in which inadvertent intraneural injection occurred, and in the ones in which large

inflammatory masses were found tracking to the knee. Those cases point out the potential hazards when a) particles are injected either near a vital or sensitive structure or b) the site of injection itself does not confine the particles to a locale effectively. Although it is probably equally likely for such events to occur with either type of particle, our histological results show an impressive advantage of LPSP over PLGA in this context: it is reasonable to contend that particles which disappear more rapidly from the tissues and that cause less long-term inflammation will be safer. Given the potentially severe sequelae of having an inflammatory mass extending into a nerve, it will probably be advisable to take special precautions (nerve stimulator, radiological guidance) when performing blocks with such particles at sites where a major nerve could be hit.

Interestingly, none of the rats injected with either type of particle showed any neurological deficits, even in the rats where there was marked inflammation, axonal degeneration, and even intraneural microspheres. The mean thermal latency prior to sacrifice was comparable to that in pristine rats. None of the rats showed any "touch-evoked agitation" or autotomy, even in the presence of marked nerve injury or perineural inflammation. This observation calls into question the usefulness of a "normal" neurobehavioural exam in assessing the presence of neurological injury, and reinforces the importance of histopathological studies in the evaluation of controlled release devices.

In summary, lipid-protein-sugar particles appear to have good long-term biocompatibility in the perineurium compared to PLGA microspheres as prepared by our fairly standard methodology. Neither type of particle caused inflammation of the nerve itself when injected in close proximity. While these results suggest that both are suitable for use in the perineurium, they do strike a note of caution regarding the injection of foreign material near sensitive structures such as nerves. The criticism has been made of several biomaterials used in medicine, that they have been adapted to biological uses even though they might not be optimal for their particular use (12). Similarly, biomaterials and polymers that are well suited for one region of the body (e.g. skin) might not be ideal in another (e.g.

near a nerve). Specifically, it does not seem ideal to have materials that cause protracted inflammation and have long tissue half-lives in proximity to important structures, particularly if the therapeutic effect is relatively short. Thus the somewhat subjective judgement of whether or not a material is biocompatible may also vary with the site of injection (e.g. a localized long-lasting inflammatory reaction would probably be felt to be more problematic in the sino-atrial node of the heart than in deep subcutaneous tissue. It may be that the concept of "biocompatibility" as a blanket clearance for use of a material anywhere in the body is dated.

REFERENCES

1. K. A. Athanasiou, G. G. Niederauer, and C. M. Agrawal. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* **17**: 93-102 (1996).
2. G. A. Brazeau, M. Sciame, S. A. al-Suwayeh, and E. Fattal. Evaluation of PLGA microsphere size effect on myotoxicity using the isolated rodent skeletal muscle model. *Pharm. Dev. Technol.* **1**: 279-83 (1996).
3. J. Castillo, J. Curley, J. Hotz, M. Uezono, J. Tigner, M. Chasin, R. Wilder, R. Langer, and C. Berde. Glucocorticoids prolong rat sciatic nerve blockade in vivo from bupivacaine microspheres. *Anesthesiology* **85**: 1157-66 (1996).
4. J. Curley, J. Castillo, J. Hotz, M. Uezono, S. Hernandez, J.-O. Lim, J. Tigner, M. Chasin, R. Langer, and C. Berde. Prolonged regional nerve blockade. Injectable biodegradable bupivacaine/polyester microspheres. *Anesthesiology* **84**: 1401-1410 (1996).
5. D. E. Cutright, J. D. Beasley, and B. Perez. Histologic comparison of polylactic and polyglycolic acid sutures. *Oral Surg.* **32**: 165-173 (1971).
6. C. Drager, D. Benziger, F. Gao, and C. B. Berde. Prolonged intercostal nerve blockade in sheep using controlled release bupivacaine and dexamethasone from polyester microspheres. *Anesthesiology* **89**: 969-979 (1998).
7. J.-P. Estebe, P. Le Corre, Y. Mallédant, F. Chevanne, and R. Leverage. Prolongation of spinal anesthesia with bupivacaine-loaded (DL-lactide) microspheres. *Anesth. Analg.* **81**: 99-103 (1995).
8. D. S. Kohane, M. Lipp, R. Kinney, N. Lotan, and R. Langer. Sciatic nerve blockade with lipid-protein-sugar particles containing bupivacaine. *Pharm. Res.* (in press) (2000).
9. P. Le Corre, P. Le Guevello, V. Gajan, F. Chevanne, and R. Le Verge. Preparation and characterization of bupivacaine-loaded polylactide and polylactide-coglycolide microspheres. *Int. J. Pharmaceut.* **107**: 41-49 (1994).

10. P. Le Corre, J. H. Rytting, V. Gajan, F. Chevanne, and R. Le Verge. *In vitro* controlled release kinetics of local anaesthetics from poly(D,L-lactide) and poly(lactide-co-glycolide) microspheres. *J. Microencaps.* **14**: 243-255 (1997).
11. P. Menei, V. Daniel, C. Montero-Menei, M. Brouillard, A. Pouplard-Barthelaix, and J. P. Benoit. Biodegradation and brain tissue reaction to poly(D,L-lactide-co-glycolide) microspheres. *Biomaterials* **14**: 470-478 (1993).
12. N. A. Peppas and R. Langer. New challenges in biomaterials. *Science* **263**: 1715-1720 (1994).
13. M. van der Elst, C. P. A. T. Klein, J. M. de Blicq-Hogervorst, P. Patka, and H. J. T. M. Haarman. Bone tissue response to biodegradable polymers used for intramedullary fracture fixation: A long-term in vivo study in sheep femora. *Biomaterials* **20**: 121-128 (1999).
14. N. Wakiyama, K. Juni, and M. Nakana. Preparation and evaluation in vitro of polylactic acid microspheres containing local anesthetics. *Chem. Pharm. Bull.* **29**: 3363-3368 (1981).
15. N. Wakiyama, K. Juni, and M. Nakano. Preparation and evaluation *in vitro* and *in vivo* of polylactic acid microspheres containing dibucaine. *Chem. Pharm. Bull.* **30**: 3719-3727 (1982).
16. P. D. Wall, M. Devor, R. Inbal, J. W. Scadding, D. Schonfeld, Z. Seltzer, and M. M. Tomkiewicz. Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. *Pain* **7**: 103-111 (1979).
17. M. Xiao, D. S. Kohane, J. Yieh, C. Irwin, L. Benowitz, and C. Berde. Prolonged sciatic nerve blockade prevents neither the rise in GAP-43 expression nor the development of pain-related behaviour following nerve crush injury. *Anesthesiology* **87**: 734A (1997).
18. A. M. Yanez, M. Wallace, R. Ho, D. Shen, and T. L. Yaksh. Touch-evoked agitation produced by spinally administered phospholipid emulsion and liposomes in rats. *Anesthesiology* **82**: 1189-1198 (1995).

Figure Legends

Fig. 1. Control Nerve. A. Low power view. Note absence of inflammatory cells outside the neural sheath (perineurium). B. High power view (400X) of connective tissue outside the perineurium. Note looseness and low cellularity of connective tissue.

Fig. 2. PLGA microspheres at the sciatic nerve. Microspheres are seen to be in close proximity to the nerve, but outside the nerve sheath. N = sciatic nerve. BV = blood vessel. M = muscle. MS = microspheres.

Fig. 3. Summary of histological findings over time. A. Dissection scores. Median \pm 25th & 75th percentiles. No statistically significant difference between groups. B. Prevalence of particle residue. C. Area of inflammation at two weeks. Median \pm 25th & 75th percentiles. In the PLGA group, the area was too large and amorphous at 4 days to be dissected en bloc. At 7 months there was no obvious entity to measure in either group. D. Cell density of inflammatory response. Mean \pm SD. In summary, there were robust differences at 2 weeks after injection.

Fig. 4. 4 days after particle injection. A. PLGA. Microspheres are surrounded by a dense infiltrate, surrounded by intense granulomatous inflammation, with some foreign body giant cells. In general (and at all time points examined), the inflammatory response to PLGA microspheres was confined to clearly demarcated pockets that were firm and slightly gritty to palpation. B. LPSPs. Dense inflammatory reaction that was more diffuse than that from PLGA. Pockets of particle were smaller and softer to the touch. In both cases, Inflammation also involved adjacent muscle and epineural adipose tissue. The injection site had necrotic muscle fibers, myophagocytosis, and myocyte regeneration – all signs of acute muscle injury.

Fig. 5. 2 weeks after particle injection. A. PLGA. The histological appearance was very similar to that at 4 days (and at 8 weeks), with lymphocytes, macrophages and giant cells in granulomas. The asterisk denotes a 'ghost' of a microsphere. One sample in this group had active myositis. B. LPSPs. There was a small loose, predominantly lymphocytic infiltrate. In both cases, histological appearance was the same for particles without drug.

Fig. 6. Ectopic particles. A. Sub-perineurial microspheres. Arrows indicate 'ghosts' of microspheres. B. Pocket of microspheres approximately 0.4 mm x 4 mm x 8 mm found at knee of rat (about 2.5 cm from site of injection).

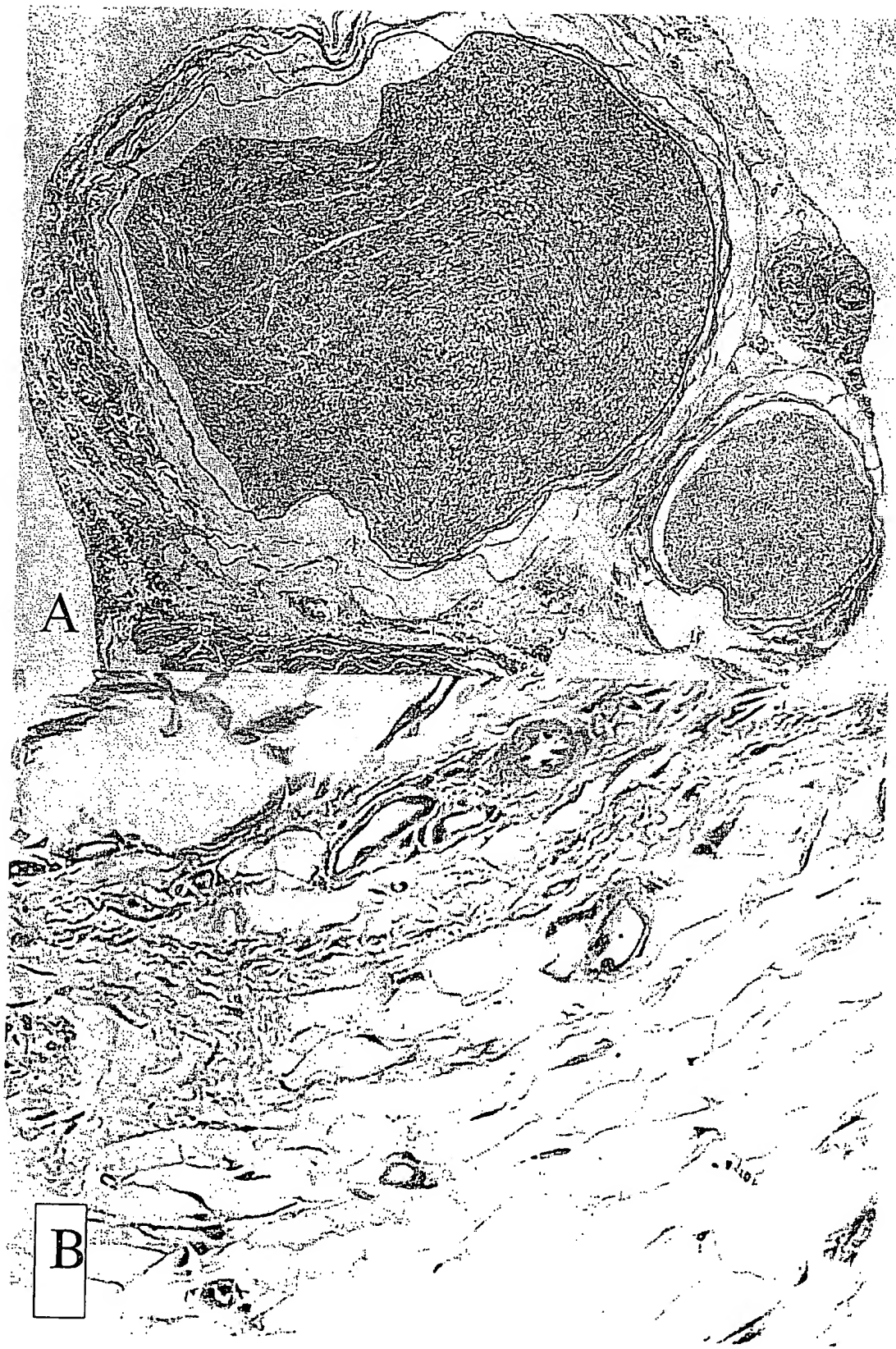


Fig 1

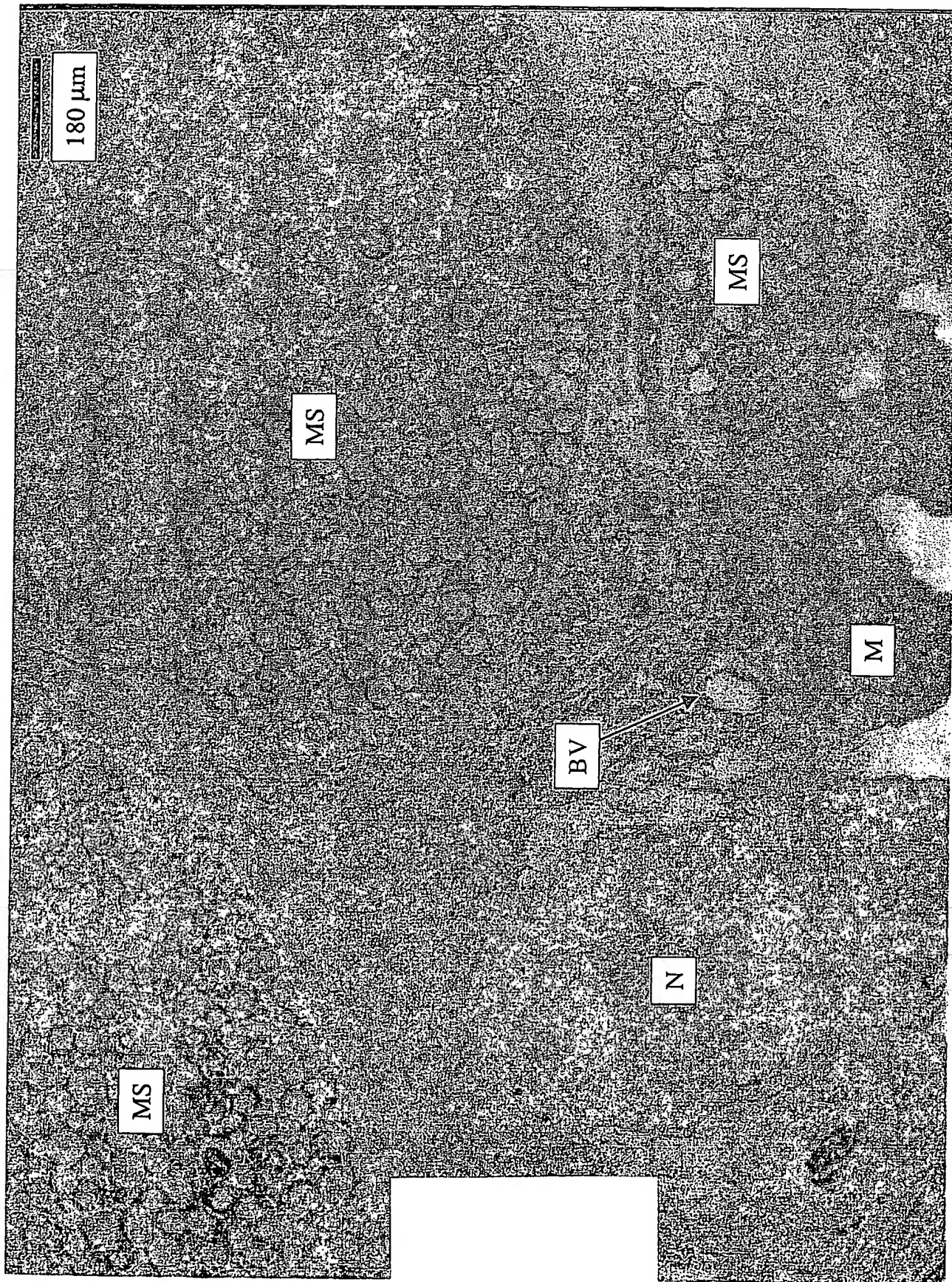
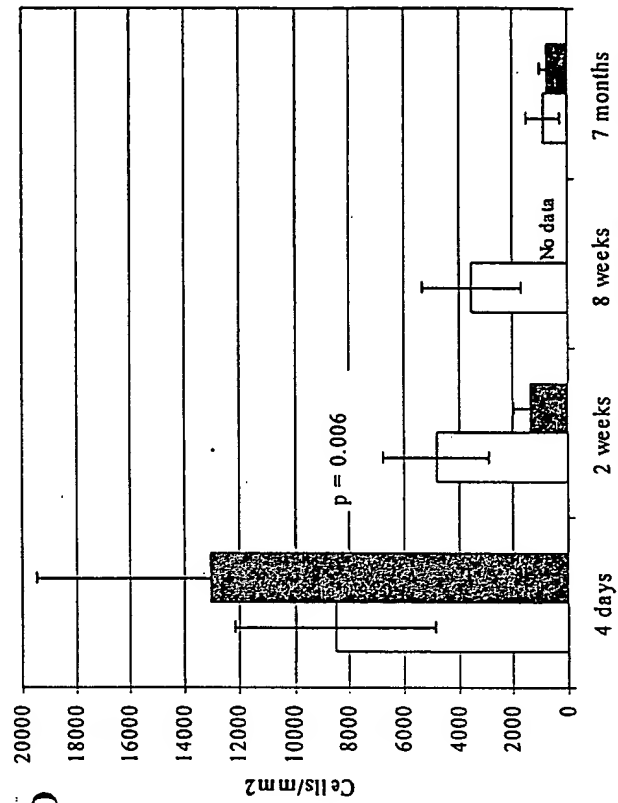
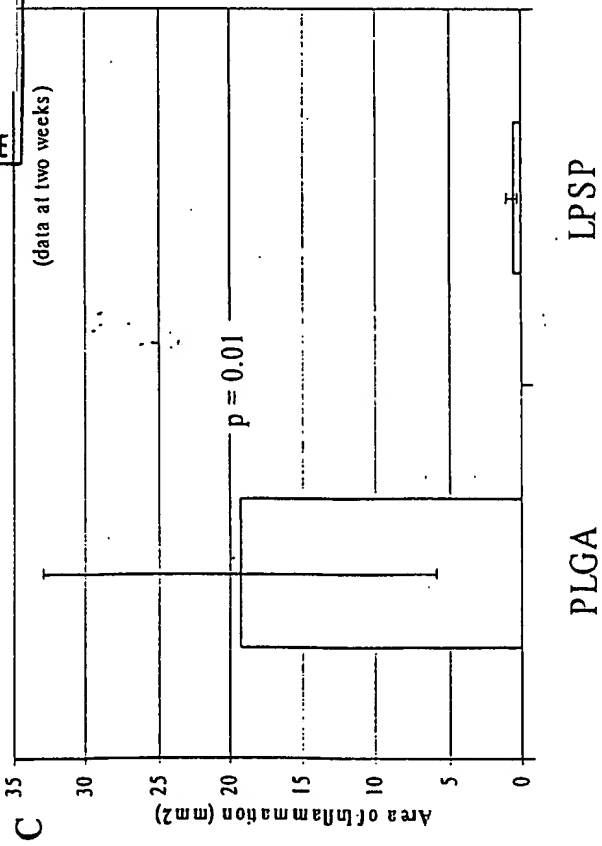
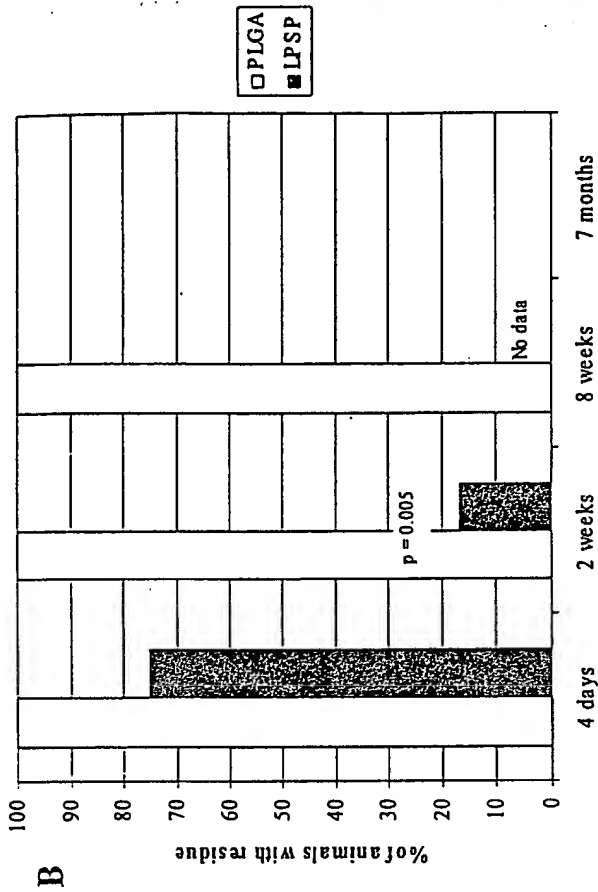
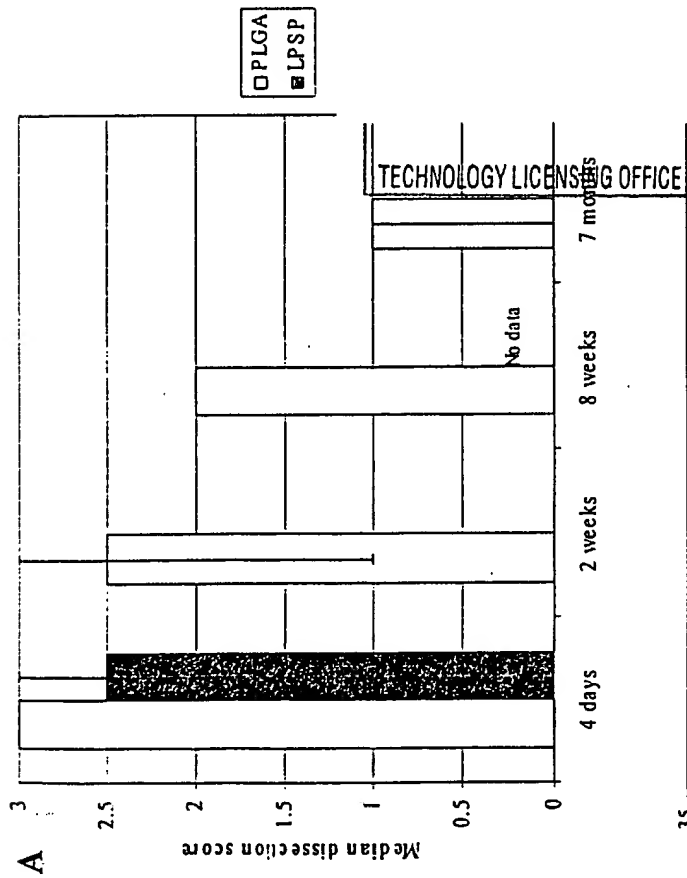


Fig. 2



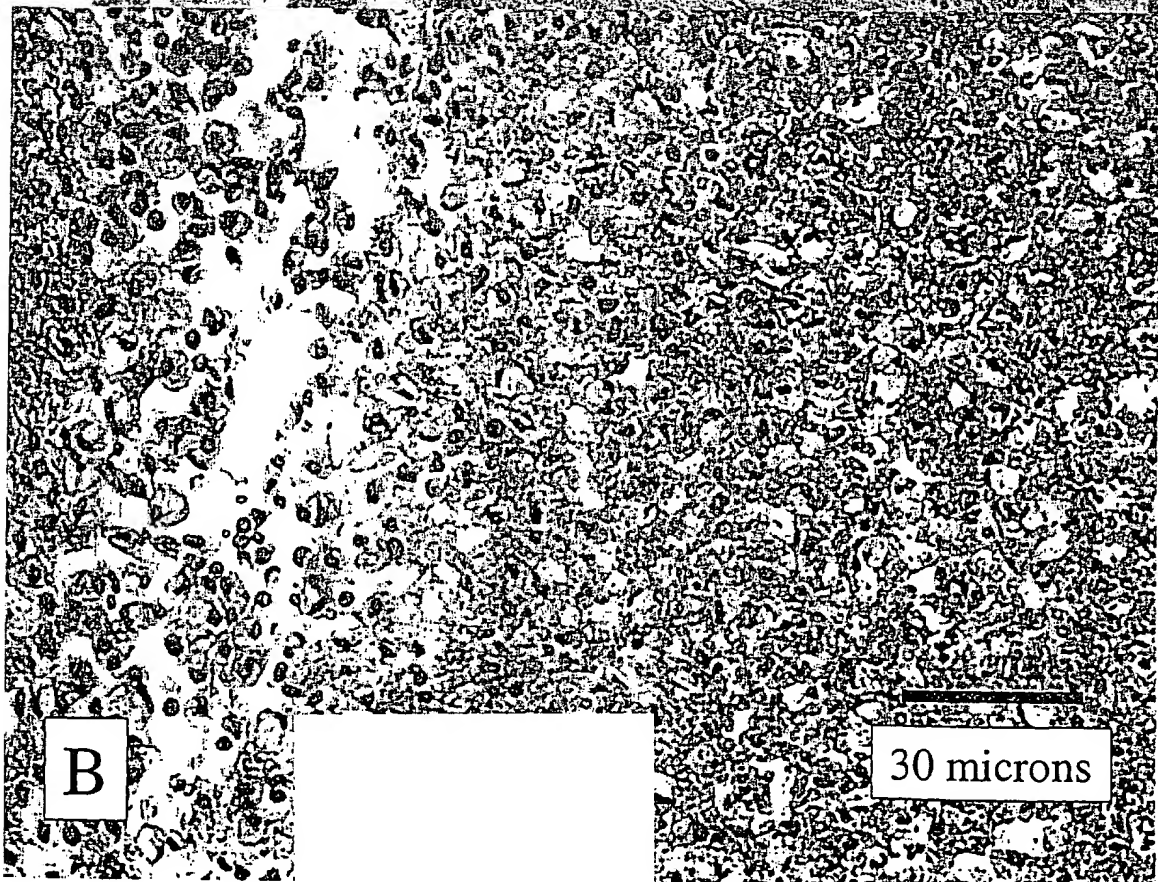
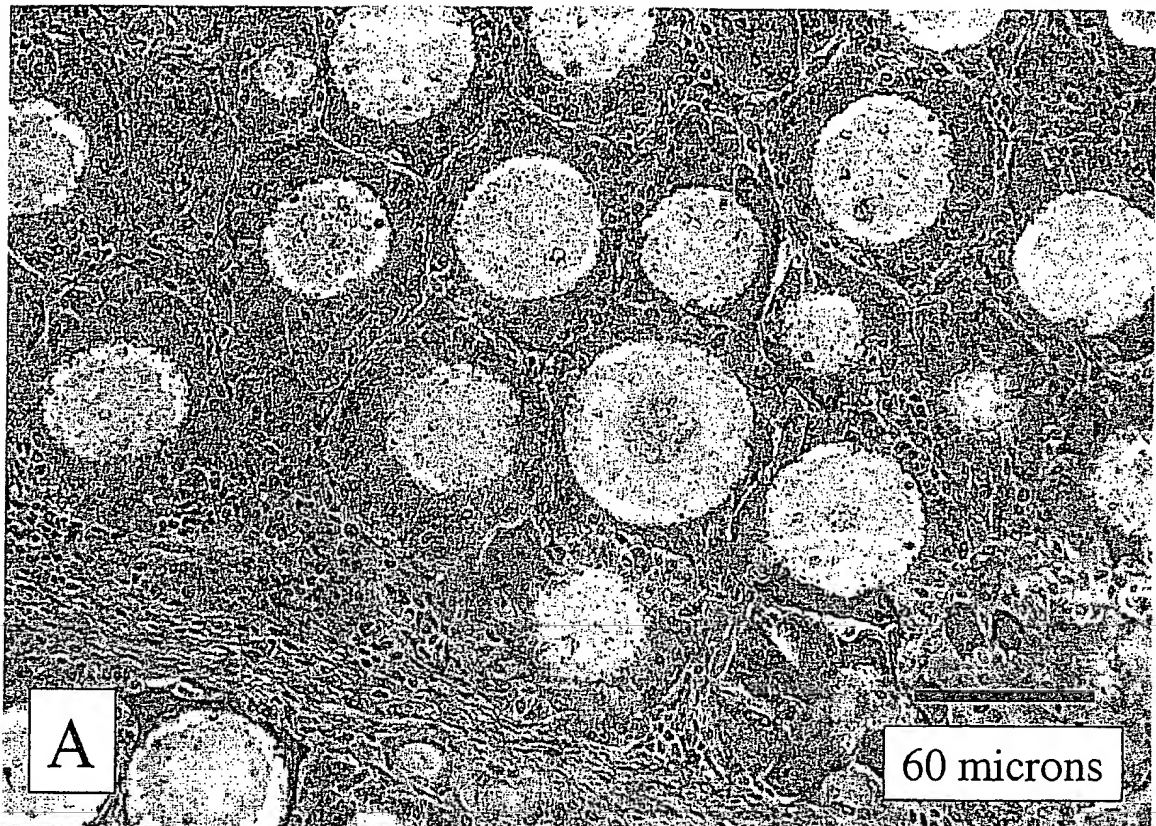


Fig 4

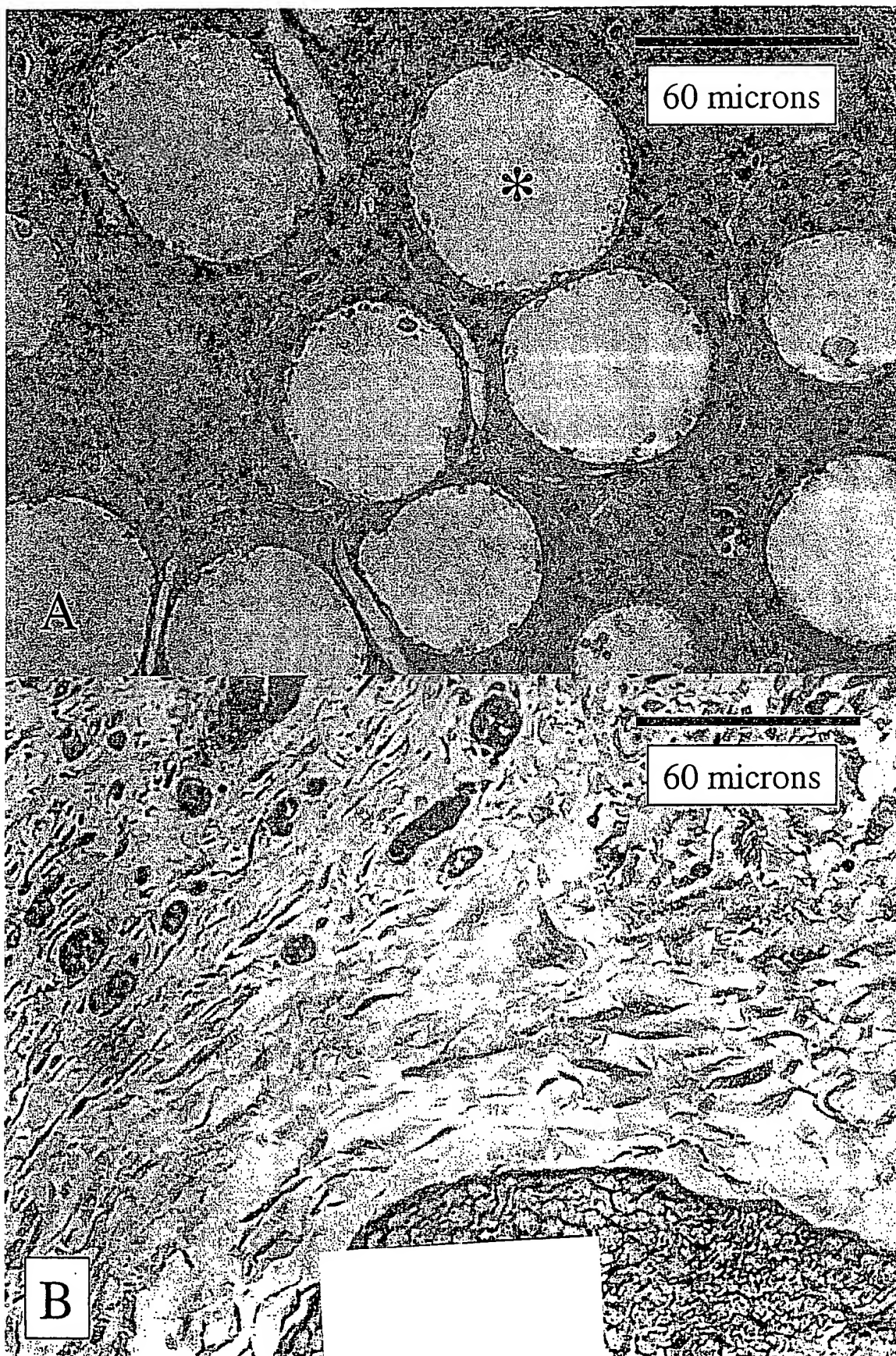


Fig 5

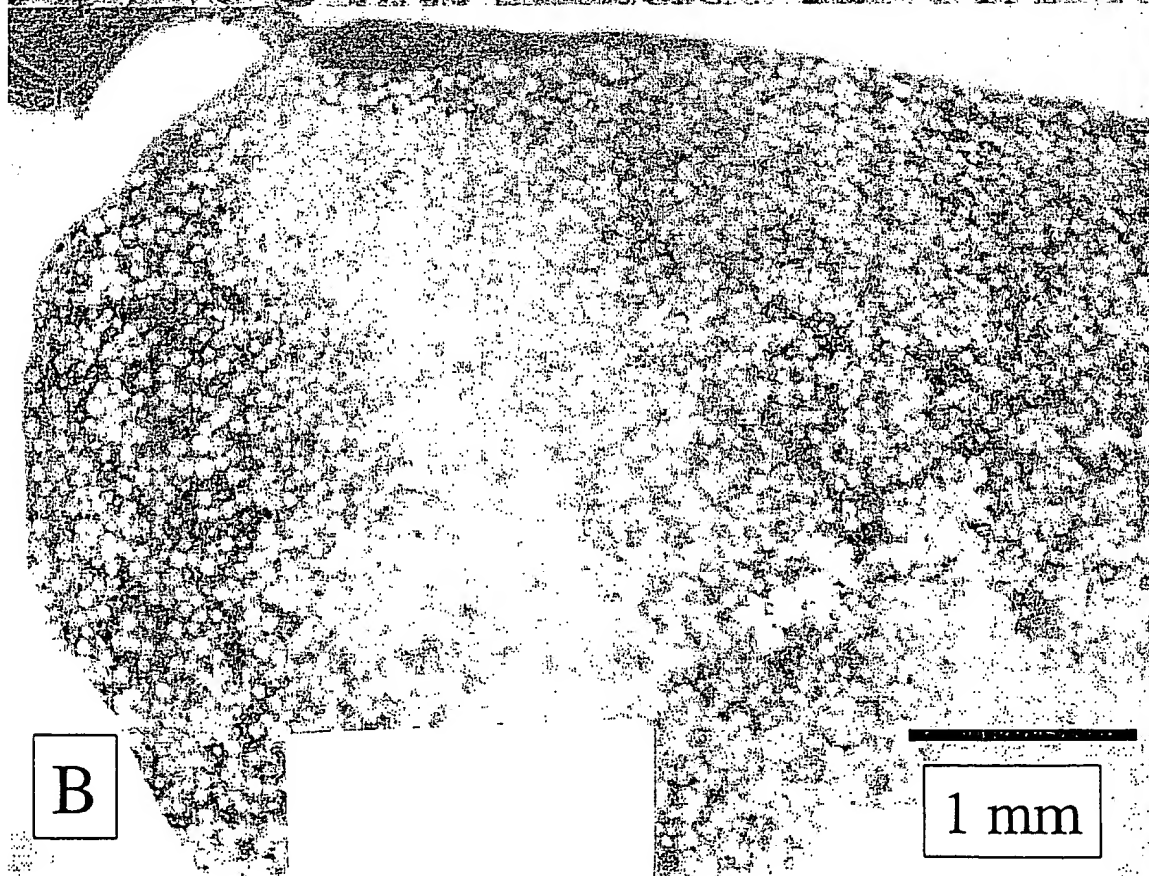
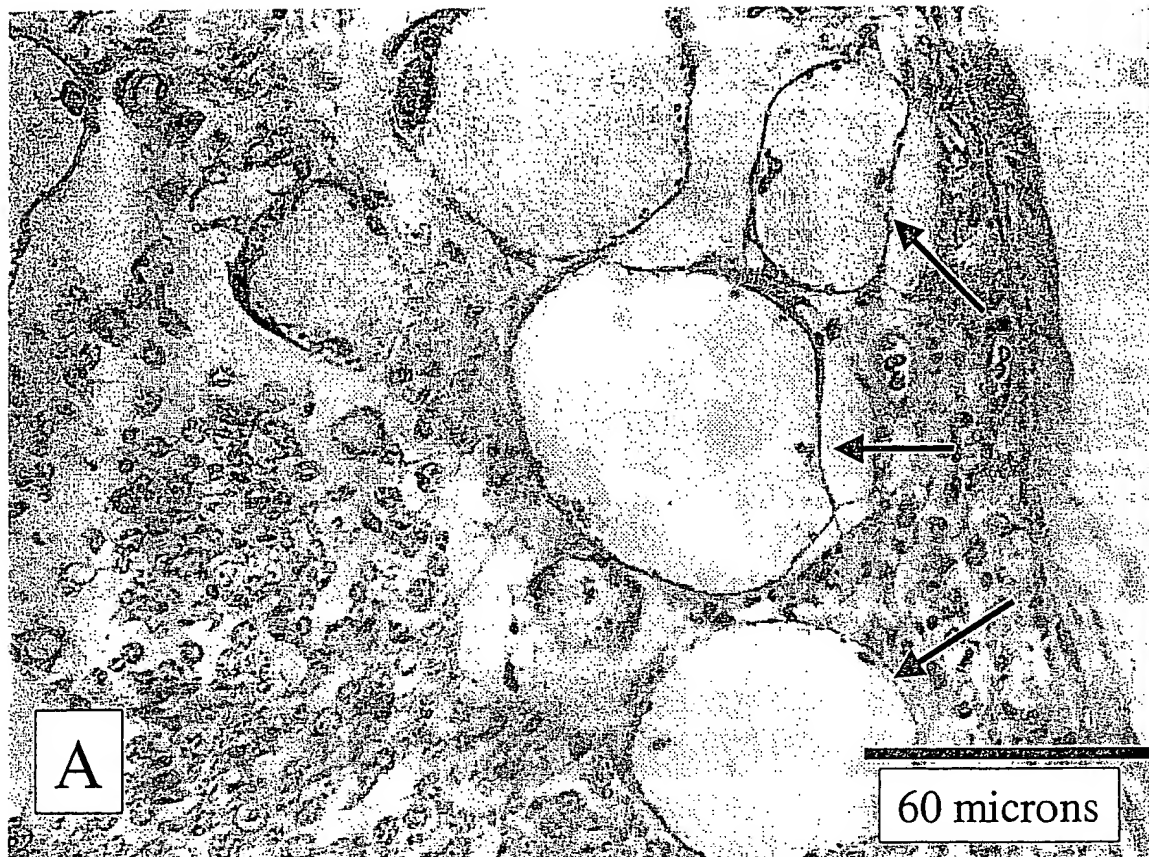


Fig 6